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DALEY *et al.*
Appl. No. 10/716,619

THE CLAIMS:

This listing will replace all prior versions and listings of claims in the application.

1-54. (Canceled)

55. (Previously presented) A serum-free, eukaryotic cell culture medium comprising the ingredients N-acetyl-L-cysteine, 2-mercaptoethanol, human serum albumin, D,L-tocopherol acetate, soluble human lipids for serum-free media, ethanolamine, human zinc insulin, iron-saturated transferrin, Se⁴⁺, hydrocortisone, Ca²⁺, K⁺, Mg²⁺, Na⁺, CO₃²⁻, PO₄³⁻, D-glucose, HEPES, sodium pyruvate, phenol red, glycine, L-alanine, L-asparagine, L-aspartic acid, L-glutamic acid, L-phenylalanine, L-histidine, L-isoleucine, L-lysine, L-leucine, L-arginine HCl, L-methionine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-valine, biotin, D-calcium pantothenate, choline chloride, folic acid, i-inositol, niacinamide, pyridoxal HCl, riboflavin, thiamine HCl, and vitamin B₁₂,

wherein each of said ingredients is present in said medium at a concentration that supports the expansion of CD34⁺ hematopoietic cells in suspension culture in the absence of stromal cells.

56-60. (Canceled)

61. (Currently Amended) A method of providing recombinant CD34⁺ hematopoietic cells to a mammal comprising:

- (a) expanding recombinant CD34⁺ hematopoietic cells according to the method of claim 60 76; and
- (b) introducing said recombinant cells into said mammal.

62-75. (Canceled)

76. (New) A method of expanding recombinant CD34⁺ hematopoietic cells, the method comprising:

- (a) contacting the cells with a serum-free medium; and

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(b) culturing the cells in serum-free suspension culture, in the absence of stromal cells, under conditions that facilitate the expansion of the cells.

77. (New) The method of claim 76, wherein the serum-free culture medium comprises N-acetyl-L-cysteine or derivatives thereof.

78. (New) The method of claim 76, wherein the serum-free culture medium comprises at least one component selected from the group consisting of 2-mercaptoethanol, human serum albumin, D,L-tocopherol acetate, soluble human lipids for serum-free media, ethanolamine, human zinc insulin, iron-saturated transferrin, Se^{4+} , hydrocortisone, Ca^{2+} , K^+ , Mg^{2+} , Na^+ , CO_3^{2-} , PO_4^{3-} , D-glucose, HEPES, sodium pyruvate, phenol red, glycine, L-alanine, L-asparagine, L-cysteine, L-aspartic acid, L-glutamic acid, L-phenylalanine, L-histidine, L-isoleucine, L-lysine, L-leucine, L-glutamine, L-arginine HCL, L-methionine, L-proline, L-hydroxyproline, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-valine, biotin, D-calcium pantothenate, choline chloride, folic acid, i-inositol, niacinamide, pyridoxal HCl, riboflavin, thiamine HCl, and vitamin B₁₂.

79. (New) The method of claim 76, wherein the serum-free culture medium comprises 2-mercaptoethanol, human serum albumin, D,L-tocopherol acetate, soluble human lipids for serum-free media, ethanolamine, human zinc insulin, iron-saturated transferrin, Se^{4+} , hydrocortisone, Ca^{2+} , K^+ , Mg^{2+} , Na^+ , CO_3^{2-} , PO_4^{3-} , D-glucose, HEPES, sodium pyruvate, phenol red, glycine, L-alanine, L-asparagine, L-aspartic acid, L-glutamic acid, L-phenylalanine, L-histidine, L-isoleucine, L-lysine, L-leucine, L-arginine HCL, L-methionine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-valine, biotin, D-calcium pantothenate, choline chloride, folic acid, i-inositol, niacinamide, pyridoxal HCl, riboflavin, thiamine HCl, and vitamin B₁₂.

80. (New) The method of claim 76, wherein the serum-free medium comprises at least one component selected from the group consisting of a trace element, a glucocorticoid, an inorganic salt, an energy source, a buffering agent, a pyruvate salt, a pH indicator, an amino acid, and a vitamin.

81. (New) The method of claim 76, wherein the serum-free medium comprises at least one cytokine or at least one growth factor.

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82. (New) The method of claim 76, wherein the serum-free medium comprises at least one glucocorticoid.

83. (New) The method of claim 82, wherein the at least one glucocorticoid is a hydrocortisone.

84. (New) The method of claim 76, wherein the cells are expanded at 37°C.

85. (New) The method of claim 76, wherein the cells are expanded for 6-8 days.

86. (New) The method of claim 76, wherein the cells are human cells.

87. (New) A method of expanding recombinant CD34⁺ hematopoietic cells in serum-free culture, the method comprising:

- (a) obtaining recombinant CD34⁺ hematopoietic cells by introducing a nucleic acid construct into CD34⁺ hematopoietic cells; and
- (b) expanding the cells in serum-free suspension culture, in the absence of stromal cells, under conditions that facilitate the expansion of the cells.

88. (New) The method of claim 87, wherein the serum-free culture medium comprises N-acetyl-L-cysteine or derivatives thereof.

89. (New) The method of claim 87, wherein the serum-free culture medium comprises at least one component selected from the group consisting of 2-mercaptoethanol, human serum albumin, D,L-tocopherol acetate, soluble human lipids for serum-free media, ethanolamine, human zinc insulin, iron-saturated transferrin, Se⁴⁺, hydrocortisone, Ca²⁺, K⁺, Mg²⁺, Na⁺, CO₃²⁻, PO₄³⁻, D-glucose, HEPES, sodium pyruvate, phenol red, glycine, L-alanine, L-asparagine, L-cysteine, L-aspartic acid, L-glutamic acid, L-phenylalanine, L-histidine, L-isoleucine, L-lysine, L-leucine, L-glutamine, L-arginine HCL, L-methionine, L-proline, L-hydroxyproline, L-serine, L-threonine, L-

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tryptophan, L-tyrosine, L-valine, biotin, D-calcium pantothenate, choline chloride, folic acid, i-inositol, niacinamide, pyridoxal HCl, riboflavin, thiamine HCl, and vitamin B₁₂.

90. (New) The method of claim 87, wherein the serum-free culture medium comprises 2-mercaptoethanol, human serum albumin, D,L-tocopherol acetate, soluble human lipids for serum-free media, ethanolamine, human zinc insulin, iron-saturated transferrin, Se⁴⁺, hydrocortisone, Ca²⁺, K⁺, Mg²⁺, Na⁺, CO₃²⁻, PO₄³⁻, D-glucose, HEPES, sodium pyruvate, phenol red, glycine, L-alanine, L-asparagine, L-aspartic acid, L-glutamic acid, L-phenylalanine, L-histidine, L-isoleucine, L-lysine, L-leucine, L-arginine HCl, L-methionine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-valine, biotin, D-calcium pantothenate, choline chloride, folic acid, i-inositol, niacinamide, pyridoxal HCl, riboflavin, thiamine HCl, and vitamin B₁₂.

91. (New) The method of claim 87, wherein the serum-free medium comprises at least one component selected from the group consisting of a trace element, a glucocorticoid, an inorganic salt, an energy source, a buffering agent, a pyruvate salt, a pH indicator, an amino acid, and a vitamin.

92. (New) The method of claim 87, wherein the serum-free medium comprises at least one cytokine or at least one growth factor.

93. (New) The method of claim 87, wherein the serum-free medium comprises at least one glucocorticoid.

94. (New) The method of claim 93, wherein the at least one glucocorticoid is a hydrocortisone.

95. (New) The method of claim 87, wherein the cells are expanded at 37°C.

96. (New) The method of claim 87, wherein the cells are expanded for 6-8 days.

97. (New) The method of claim 87, wherein the cells are human cells.